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(54) Title: ANTI-TNF α ANTIBODIES IN THERAPY OF ASTHMA (57) Abstract The present invention provides for uses of an anti-TNF α antibody or an antigen-binding fragment thereof for the manufacture of a medicament for use in the treatment of asthma or airway inflammation in an individual in need thereof. The present invention also provides for use of an anti-TNF α antibody or an antigen-binding fragment thereof for the manufacture of a medicament of use in reducing accumulation in lungs of inflammatory cells in an individual in need thereof.		

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ANTI-TNF α ANTIBODIES IN THERAPY OF ASTHMA

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application No. 09/465,691, filed December 17, 1999, which is a continuation of U.S. Application No. 09/260,953, filed March 2, 1999. The entire teachings of these applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Asthma is a chronic inflammatory disorder of the airways which usually presents in the form of recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible, either spontaneously or with treatment.

Many cells and cellular elements play a role in the airway inflammation, in particular, mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils and epithelial cells. The inflammation is associated with plasma exudation, oedema, smooth muscle hypertrophy, mucus plugging and epithelial changes. The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli.

Variable airflow obstruction and bronchial hyperactivity (both specific and nonspecific) are central features in symptomatic asthma. Inflammation of the airway leads to contraction of airway smooth muscle, microvascular leakage and bronchial hyperresponsiveness. When airway reactivity is high, symptoms are more severe and persistent and the magnitude of diurnal fluctuations in lung function is greater. The mechanism by which airway inflammation is related to bronchial reactivity is unclear. Recent research indicates that tumor necrosis factor alpha (TNF α), which is expressed in increased amounts in asthmatic airways, maybe associated with the increased airway hyperresponsiveness (Shah *et al.*, *Clin. Exper. Allergy*, 25:1038-1044 (1995)). For example, intravenous administration of recombinant

TNF α to sheep resulted in marked accentuation in histamine induced airway reactivity (Wheeler *et al.*, *J. Appl. Physiol.*, 68:2542-2549 (1990)) while exposure of rats to aerosolized TNF α increased airway hyperreponsiveness and induced a minor degree of airway inflammation (Kips *et al.*, *Am. Rev. Respir. Dis.*, 145:332-336 (1992)). In normal human subjects, inhalation of recombinant TNF α caused increased bronchial reactivity (Yates *et al.*, *Thorax*, 48:1080 (1993)), while immunohistochemical analysis of bronchial biopsies from mild allergic asthmatics revealed that the increase in TNF α immunoreactivity correlated with airway hyperresponsiveness (Hosselet *et al.*, *Am. J. Respir. Crit. Care Med.*, 149:A957 (1994)).

Asthma is very common. It affects nearly 5% of the population in industrialized nations, yet it is underdiagnosed and undertreated. There is evidence that the incidence and prevalence of asthma are rising. These trends are occurring despite increases in the available therapies for asthma, which suggests that current methods of treating asthma are inadequate or not being utilized appropriately.

SUMMARY OF THE INVENTION

The present invention relates to the discovery that the clinical signs and symptoms associated with asthma can be ameliorated by treatment with an anti-TNF α antibody. As a result, the present invention provides uses of an anti-TNF α antibody or an antigen-binding fragment thereof for the manufacture of a medicament for use in the treatment of asthma or airway inflammation, e.g., as associated with asthma, in an individual in need thereof. The present invention also provides for use of an anti-TNF α antibody or an antigen-binding fragment thereof for the manufacture of a medicament for use in reducing accumulation in lungs of inflammatory cells, e.g., as associated with asthma, in an individual in need thereof. In a preferred embodiment, the antibody is a chimeric antibody such as the cA2 monoclonal antibody.

The present invention also provides methods of treating asthma or airway inflammation, e.g., as associated with asthma, in an individual comprising

administering to the individual a therapeutically effective amount of an anti-TNF α antibody or an antigen-binding fragment thereof. The invention further provides methods of reducing accumulation in lungs of inflammatory cells, e.g., as associated with asthma, in an individual in need thereof.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph showing bronchoalveolar lavage (BAL) fluid inflammatory cell accumulation (total accumulation and eosinophil accumulation) at 72 hours following ovalbumin (OA; 5% for 20 minutes) or saline (n=10) challenge in sensitized mice treated intravenously 1 hour prior to and 24 and 48 hours following OA challenge with either (1) vehicle (PBS, n=10), (2) cV1qmuG2a antibody (1 mg/kg, n=10) or (3) cV1q muG2a antibody (10 mg/kg, n=9). An additional group of 10 mice were treated intraperitoneally 1 hour prior to and 24 and 48 hours following OA challenge with dexamethasone at 1 mg/kg. * indicates statistically significant ($p < 0.05$) difference compared to the vehicle-treated group.

Figure 2 is a bar graph showing BAL fluid eosinophil accumulation at 72 hours following OA (5% for 20 minutes) or saline (n=10) challenge in sensitized mice treated intravenously 1 hour prior to and 24 and 48 hours following OA challenge with either (1) vehicle (PBS, n=10), (2) cV1qmuG2a antibody (1 mg/kg, n=10) or (3) cV1qmuG2a antibody (10 mg/kg, n=9). An additional group of 10 mice were treated intraperitoneally 1 hour prior to and 24 and 48 hours following OA challenge with dexamethasone at 1 mg/kg. Values are presented as a % of total cells mean \pm SEM. * indicates statistically significant ($p < 0.05$) difference compared to the vehicle-treated group.

Figure 3 is a bar graph showing total serum IgE at 72 hours following OA (5% for 20 minutes) or saline (n=10) challenge in sensitized mice treated intravenously 1 hour prior to and 24 and 48 hours following OA challenge with either (1) vehicle (PBS, n=10), (2) cV1q muG2a antibody (1 mg/kg, n=10) or (3) cV1qmuG2a antibody (10 mg/kg, n=9). An additional group of 10 mice were treated intraperitoneally 1 hour prior to and 24 and 48 hours following OA challenge with dexamethasone at 1 mg/kg.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the unexpected and surprising discovery that the accumulation in lungs of inflammatory cells associated with asthma, particularly bronchoalveolar lavage (BAL) eosinophils, perivascular leukocytes, interstitial
5 leukocytes and pleural leukocytes, is significantly reduced with treatment with an anti-TNF α antibody. Airway infiltration by inflammatory cells, particularly of eosinophils into the lungs, is one of the characteristic features of asthma (Holgate, *Eur. Respir. J.*, 6:1507-1520 (1993)). Bronchial biopsy studies performed in patients with allergic asthma show that increased numbers of eosinophils and
10 activated T lymphocytes are present in airway tissue and BAL.

The numbers of eosinophils in peripheral blood and BAL fluid have been shown to correlate with both the degree of bronchial hyperreactivity and asthma severity (Corrigan and Kay, *Immunology Today*, 13:501-507 (1992)). Eosinophils store four basic proteins in their granules: major basic protein, eosinophil-derived
15 neurotoxin, eosinophil cationic protein and eosinophil peroxidase. The release of these proteins may be responsible for airway tissue damage and bronchial hyperresponsiveness in asthmatics (Flavahan *et al.*, *Am. Rev. Respir. Dis.*, 138:685-688 (1988)).

T lymphocytes produce cytokines that activate cell-mediated immunity as
20 well as humoral (IgE) immune responses. Allergic asthma is dependent on an IgE response controlled by T and B lymphocytes and activated by the interaction of antigen with mast cell-bound IgE molecules.

The results described herein demonstrate that therapy with anti-TNF α antibody is beneficial in treating asthma or airway inflammation. The results herein
25 demonstrate that clinical signs and symptoms associated with asthma can be ameliorated by treatment with an anti-TNF α antibody. As a result, the present invention provides methods of treating asthma or airway inflammation in an individual comprising administering an anti-TNF α antibody or an antigen-binding fragment of the anti-TNF α antibody to the individual. In a particular embodiment,
30 the invention provides methods of treating airway inflammation associated with

asthma. The invention also provides methods of reducing accumulation in lungs of inflammatory cells in an individual in need thereof. In a particular embodiment, the invention provides methods of reducing accumulation in lungs of inflammatory cells associated with asthma. Symptoms, as used herein, refer to subjective feelings. For example, symptoms include when a patient complains of breathlessness, chest tightness, insomnia. Signs, as used herein, refer to what is objectively observed. For example, signs include the results of pulmonary and other laboratory tests.

Tumor Necrosis Factor Alpha

TNF α is a soluble homotrimer of 17 kD protein subunits (Smith *et al.*, *J. Biol. Chem.*, 262:6951-6954 (1987)). A membrane-bound 26 kD precursor form of TNF α also exists (Kriegler *et al.*, *Cell*, 53:45-53 (1988)). For reviews of TNF α , see Beutler *et al.*, *Nature*, 320(6063):584-588 (1986); Old, *Science*, 230:630-632 (1986); and Le *et al.*, *Lab. Invest.*, 56:234 (1987).

TNF α is produced by a variety of cells including monocytes and macrophages, lymphocytes, particularly cells of the T cell lineage (Vassalli, *Annu. Rev. Immunol.*, 10:411-452 (1992)), neutrophils (Dubravec *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6758-6761 (1990)), epithelial cells (Ohkawara *et al.*, *Am. J. Respir. Cell. Biol.*, 7:985-392 (1992)) and mast cells (Shah *et al.*, *Clin. Exper. Allergy*, 25:1038-1044 (1995); Gordon *et al.*, *Nature*, 346:274-276 (1990); Gordon *et al.*, *J. Exp. Med.*, 174:103-107 (1991); Bradding *et al.*, *Am. J. Respir. Cell. Mol. Biol.*, 10:471-480 (1994); Walsh *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:4220-4224 (1991); Benyon *et al.*, *J. Immunol.*, 147:2253-2258 (1991); and Ohkawara *et al.*, *Am. J. Respir. Cell. Biol.*, 7:985-392 (1992)). Eosinophils have also been suggested as a source of TNF α (Costa *et al.*, *J. Clin. Invest.*, 91:2673-2684 (1993)).

Anti-TNF α Antibodies

As used herein, an anti-tumor necrosis factor alpha antibody decreases, blocks, inhibits, abrogates or interferes with TNF α activity *in vivo*. In a preferred embodiment, the antibody specifically binds the antigen. The antibody can be polyclonal or monoclonal, and the term antibody is intended to encompass both

polyclonal and monoclonal antibodies. The terms polyclonal and monoclonal refer to the degree of homogeneity of an antibody preparation, and are not intended to be limited to particular methods of production. Single chain antibodies, and chimeric, humanized or primatized (CDR-grafted antibodies, with or without framework changes), or veneered antibodies, as well as chimeric, CDR-grafted or veneered single chain antibodies, comprising portions derived from different species, and the like are also encompassed by the present invention and the term "antibody".

In a particular embodiment, the anti-TNF α antibody is a chimeric antibody. In a preferred embodiment, the anti-TNF α antibody is chimeric monoclonal antibody cA2 (or an antigen binding fragment thereof) or murine monoclonal antibody A2 (or an antigen binding fragment thereof), or has an epitopic specificity similar to that of chimeric antibody cA2, murine monoclonal antibody A2, or antigen binding fragments thereof, including antibodies or antigen binding fragments reactive with the same or a functionally equivalent epitope on human TNF α as that bound by chimeric antibody cA2 or murine monoclonal antibody A2, or antigen binding fragments thereof. Antibodies with an epitopic specificity similar to that of chimeric antibody cA2 or murine monoclonal antibody A2 include antibodies which can compete with chimeric antibody cA2 or murine monoclonal antibody A2 (or antigen binding fragments thereof) for binding to human TNF α . Such antibodies or fragments can be obtained as described above. Chimeric antibody cA2, murine monoclonal antibody A2 and methods of obtaining these antibodies are also described in Le *et al.*, U.S. Patent No. 5,656,272; Le *et al.*, U.S. Patent No. 5,698,195; U.S. Application No. 08/192,093 (filed February 4, 1994); U.S. Patent No. 5,919,452; Le, J. *et al.*, International Publication No. WO 92/16553 (published October 1, 1992); Knight, D.M. *et al.*, *Mol. Immunol.*, 30:1443-1453 (1993); and Siegel, S.A. *et al.*, *Cytokine*, 7(1):15-25 (1995), which references are each entirely incorporated herein by reference. Chimeric antibody cA2 is also known as infliximab and REMICADE.

Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNF α IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human

IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric antibody cA2 is derived from the variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line.

Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNF α in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant human TNF α , the affinity constant of chimeric antibody cA2 was calculated to be $1.04 \times 10^{10} \text{M}^{-1}$. Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988; Colligan *et al.*, eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992, 1993); Kozbor *et al.*, *Immunol. Today*, 4:72-79 (1983); Ausubel *et al.*, eds. *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987, 1992, 1993); and Muller, *Meth. Enzymol.*, 92:589-601 (1983), which references are entirely incorporated herein by reference.

In a particular embodiment, chimeric antibody cA2 is produced by a cell line designated c168A and murine monoclonal antibody A2 is produced by a cell line designated c134A.

Additional examples of anti-TNF α antibodies (or antigen-binding fragments thereof) are described in the art (see, e.g., U.S. Patent No. 5,231,024; Möller, A. *et al.*, *Cytokine*, 2(3):162-169 (1990); U.S. Application No. 07/943,852 (filed September 11, 1992); Rathjen *et al.*, International Publication No. WO 91/02078 (published February 21, 1991); Rubin *et al.*, EPO Patent Publication No. 0 218 868 (published April 22, 1987); Yone *et al.*, EPO Patent Publication No. 0 288 088 (October 26, 1988); Liang, *et al.*, *Biochem. Biophys. Res. Comm.*, 137:847-854 (1986); Meager, *et al.*, *Hybridoma*, 6:305-311 (1987); Fendly *et al.*, *Hybridoma*, 6:359-369 (1987); Bringman, *et al.*, *Hybridoma*, 6:489-507 (1987); and Hirai, *et al.*,

J. Immunol. Meth., 96:57-62 (1987), which references are entirely incorporated herein by reference).

Suitable antibodies are available, or can be raised against an appropriate immunogen, such as isolated and/or recombinant antigen or portion thereof (including synthetic molecules, such as synthetic peptides) or against a host cell which expresses recombinant antigen. In addition, cells expressing recombinant antigen, such as transfected cells, can be used as immunogens or in a screen for antibody which binds receptor (see e.g., Chuntharapai *et al.*, *J. Immunol.*, 152: 1783-1789 (1994); and Chuntharapai *et al.*, U.S. Patent No. 5,440,021).

Preparation of immunizing antigen, and polyclonal and monoclonal antibody production can be performed using any suitable technique. A variety of methods have been described (see e.g., Kohler *et al.*, *Nature*, 256: 495-497 (1975) and *Eur. J. Immunol.*, 6: 511-519 (1976); Milstein *et al.*, *Nature*, 266: 550-552 (1977); Koprowski *et al.*, U.S. Patent No. 4,172,124; Harlow, E. and D. Lane, 1988, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory: Cold Spring Harbor, NY); and *Current Protocols In Molecular Biology*, Vol. 2 (Supplement 27, Summer '94), Ausubel *et al.*, Eds., (John Wiley & Sons: New York, NY), Chapter 11, (1991)). Generally, a hybridoma can be produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as SP2/0) with antibody producing cells. The antibody producing cell, preferably those of the spleen or lymph nodes, can be obtained from animals immunized with the antigen of interest. The fused cells (hybridomas) can be isolated using selective culture conditions, and cloned by limiting dilution. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Other suitable methods of producing or isolating antibodies of the requisite specificity, including human antibodies, can be used, including, for example, methods by which a recombinant antibody or portion thereof are selected from a library, such as, for example, by phage display technology (see, e.g., Winters *et al.*, *Annu. Rev. Immunol.*, 12:433-455 (1994); Hoogenboom *et al.*, WO 93/06213; Hoogenboom *et al.*, U.S. Patent No. 5,565,332; WO 94/13804, published June 23, 1994; Krebber *et al.*, U.S. Patent No. 5,514,548; and Dower *et al.*, U.S. Patent

No. 5,427,908), or which rely upon immunization of transgenic animals (e.g., mice) capable of producing a full repertoire of human antibodies (see e.g., Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 2551-2555 (1993); Jakobovits *et al.*, *Nature*, 362: 255-258 (1993); Kucherlapati *et al.*, European Patent No. EP 0 463 151 B1; 5 Lonberg *et al.*, U.S. Patent No. 5,569,825; Lonberg *et al.*, U.S. Patent No. 5,545,806; and Surani *et al.*, U.S. Patent No. 5,545,807).

The various portions of single chain antibodies, chimeric, humanized or primatized (CDR-grafted antibodies, with or without framework changes), or veneered antibodies, as well as chimeric, CDR-grafted or veneered single chain 10 antibodies, comprising portions derived from different species, can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., Cabilly *et al.*, U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent No. 15 0,125,023 B1; Boss *et al.*, U.S. Patent No. 4,816,397; Boss *et al.*, European Patent No. 0,120,694 B1; Neuberger, M.S. *et al.*, WO 86/01533; Neuberger, M.S. *et al.*, European Patent No. 0,194,276 B1; Winter, U.S. Patent No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Queen *et al.*, U.S. Patent No. 5,585,089; Queen *et al.*, European Patent No. 0,451,216 B1; Adair *et al.*, WO 91/09967, published 11 20 July 1991; Adair *et al.*, European Patent No. 0,460,167 B1; and Padlan, E.A. *et al.*, European Patent No. 0,519,596 A1. See also, Newman, R. *et al.*, *BioTechnology*, 10: 1455-1460 (1992), regarding primatized antibody, and Huston *et al.*, U.S. Patent No. 5,091,513; Huston *et al.*, U.S. Patent No. 5,132,405; Ladner *et al.*, U.S. Patent No. 4,946,778 and Bird, R.E. *et al.*, *Science*, 242: 423-426 (1988)) regarding single 25 chain antibodies.

In addition, antigen binding fragments of antibodies, including fragments of chimeric, humanized, primatized, veneered or single chain antibodies and the like, can also be produced. For example, antigen binding fragments include, but are not limited to, fragments such as Fv, Fab, Fab' and F(ab')₂ fragments. Antigen binding 30 fragments can be produced by enzymatic cleavage or by recombinant techniques, for example. For instance, papain or pepsin cleavage can generate Fab or F(ab')₂

fragments, respectively. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons has been introduced upstream of the natural stop site. For example, a chimeric gene encoding a F(ab')₂ heavy chain portion can be designed to include DNA sequences encoding the CH₁ domain and hinge region of the heavy chain.

Anti-TNF α antibodies suitable for use in the present invention are characterized by high affinity binding to TNF α and low toxicity (including human anti-murine antibody (HAMA) and/or human anti-chimeric antibody (HACA) response). An antibody where the individual components, such as the variable region, constant region and framework, individually and/or collectively possess low immunogenicity is suitable for use in the present invention. Antibodies which can be used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, may contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HACA or HAMA responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titers in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (see, e.g., Elliott *et al.*, *Lancet* 344:1125-1127 (1994), incorporated herein by reference).

As used herein, the term "antigen binding region" refers to that portion of an antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. The antigen binding region includes the "framework" amino acid residues necessary to maintain the proper conformation of the antigen-binding residues.

The term antigen refers to a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of selectively binding to an epitope of that antigen. An antigen can have one or more than one epitope.

The term epitope is meant to refer to that portion of the antigen capable of being recognized by and bound by an antibody at one or more of the antibody's

antigen binding region. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics. By "inhibiting and/or neutralizing epitope" is intended an epitope, which, when
5 bound by an antibody, results in loss of biological activity of the molecule containing the epitope, *in vivo* or *in vitro*, more preferably *in vivo*, including binding of TNF α to a TNF α receptor.

Administration

Anti-TNF α antibodies can be administered to a patient in a variety of ways.
10 In a preferred embodiment, anti-TNF α antibodies are administered by inhalation (e.g., in an inhalant or spray or as a nebulized mist). Other routes of administration include intranasal, oral, intravenous including infusion and/or bolus injection, intradermal, transdermal (e.g., in slow release polymers), intramuscular, intraperitoneal, subcutaneous, topical, epidural, buccal, etc. routes. Other suitable
15 routes of administration can also be used, for example, to achieve absorption through epithelial or mucocutaneous linings. Antibodies can also be administered by gene therapy, wherein a DNA molecule encoding a particular therapeutic protein or peptide is administered to the patient, e.g., via a vector, which causes the particular protein or peptide to be expressed and secreted at therapeutic levels *in vivo*. In
20 addition, anti-TNF α antibodies can be administered together with other components of biologically active agents, such as pharmaceutically acceptable surfactants (e.g., glycerides), excipients (e.g., lactose), carriers, diluents and vehicles. If desired, certain sweetening, flavoring and/or coloring agents can also be added.

Anti-TNF α antibodies can be administered prophylactically or
25 therapeutically to an individual prior to, simultaneously with or sequentially with other therapeutic regimens or agents (e.g., multiple drug regimens). Anti-TNF α antibodies that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions.

Anti-TNF α antibodies can be formulated as a solution, suspension, emulsion
30 or lyophilized powder in association with a pharmaceutically acceptable parenteral

vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical
5 stability (e.g., buffers and preservatives). The formulation can be sterilized by commonly used techniques. In a preferred embodiment, anti-TNF α antibodies are administered via the intranasal route (by inhalation). Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences.

A "therapeutically effective amount" of anti-TNF α antibody or antigen-
10 binding fragment is defined herein as that amount, or dose, of anti-TNF α antibody or antigen-binding fragment that, when administered to an individual, is sufficient for therapeutic efficacy (e.g., an amount sufficient for significantly reducing or eliminating symptoms or signs, or both symptoms and signs, associated with asthma or airway inflammation). The dosage administered to an individual will vary
15 depending upon a variety of factors, including the pharmacodynamic characteristics of the particular anti-TNF α antibody, and its mode and route of administration; size, age, sex, health, body weight and diet of the recipient; nature and extent of symptoms of the disease or disorder being treated, kind of concurrent treatment, frequency of treatment, and the effect desired.

20 The therapeutically effective amount can be administered in single or divided doses (e.g., a series of doses separated by intervals of days, weeks or months), or in a sustained release form, depending upon factors such as nature and extent of symptoms, kind of concurrent treatment and the effect desired. Other therapeutic regimens or agents can be used in conjunction the present invention. Adjustment
25 and manipulation of established dosage ranges are well within the ability of those skilled in the art.

Once a therapeutically effective amount has been administered, a maintenance amount of anti-TNF α antibody can be administered to the individual. A maintenance amount is the amount of anti-TNF α antibody necessary to maintain
30 the reduction or elimination of symptoms and/or signs achieved by the therapeutically effective dose. The maintenance amount can be administered in the

form of a single dose, or a series of doses separated by intervals of days or weeks (divided doses).

Second or subsequent administrations can be administered at a dosage which is the same, less than or greater than the initial or previous dose administered to the individual. A second or subsequent administration is preferably during or immediately prior to relapse or a flare-up of the disease or symptoms of the disease. For example, the second and subsequent administrations can be given between about one day to 30 weeks from the previous administration. Two, three, four or more total administrations can be delivered to the individual, as needed.

Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The present invention will now be illustrated by the following Examples, which are not intended to be limiting in any way.

EXAMPLES

EXAMPLE 1 Effects Of A Monoclonal Anti-TNF α Antibody In A Mouse Model For Allergic Asthma.

The mouse is a standard species used in pulmonary pharmacology studies. The murine model for allergic asthma used in the experiments described herein mimics human asthma in its phenotypic characteristics. In particular, both diseases are characterized by peribronchial inflammatory cell infiltration, particularly an influx of eosinophils into lungs. Thus, the mouse model serves as a good approximation to human disease.

Anti-TNF α Antibody

The anti-TNF α antibody cV1q muG2a was constructed by Centocor, Inc. (Malvern, PA). Hybridoma cells secreting the rat anti-murine TNF α antibody V1q were from Peter Krammer at the German Cancer Research Center, Heidelberg, Germany (Echtenacher *et al.*, *J. Immunol.* 145:3762-3766 (1990)). Genes encoding the variable regions of the heavy and light chains of the V1q antibody were cloned. The cloned heavy chain was inserted into four different gene expression vectors to encode cV1q heavy chain with either a human IgG1, human IgG3, murine IgG1 or murine IgG2a constant region. The V1q light chain gene was inserted into other expression vectors to encode either a human kappa or a murine kappa light chain constant region.

SP2/0 myeloma cells were transfected with the different heavy and light chain gene constructs. Cell clones producing chimeric V1q (cV1q) antibody were identified by assaying cell supernatant for human or murine IgG using standard ELISA assays. High-producing clones were subcloned to obtain homogenous cell lines. The murine IgG1 and IgG2a versions are referred to as C257A and C258, respectively. cV1q antibody was purified from cell supernatant by protein A chromatography.

cV1q antibody was characterized by measuring its affinity for soluble murine TNF α , testing its ability to protect WEHI cells from murine TNF α cytotoxicity, examining its ability to neutralize or bind murine lymphotoxin, comparing the ability of the murine IgG1 and IgG2a versions to trigger complement-mediated lysis of cells expressing recombinant transmembrane murine TNF α , and examining the ability of the human IgG1 version to protect mice from lethal doses of LPS (endotoxin). cV1q binds murine TNF (muTNF) with high affinity, neutralizes muTNF in a WEHI cell cytotoxicity assay, triggers an isotype-dependent fashion complement-mediated cytotoxicity of cells expressing transmembrane muTNF. Further, cV1q did not neutralize murine lymphotoxin cytotoxic activity. The murine IgG2a version of cV1q antibody was used in the following experimental procedure, and is referred to herein as cV1q muG2a antibody.

Experimental Procedure

Fifty female Balb/CJ mice, weighing 15-23 grams, were sensitized at 7 weeks of age by intraperitoneal injections of 10 µg ovalbumin (OA; Sigma Chemical Co., St. Louis, MO) mixed in 1.6 mg aluminum hydroxide gel suspension (Intergen, Inc., Purchase, NY) in 0.2 ml sterile saline on days 0, 7 and 14. This suspension was prepared one hour before intraperitoneal injection into each mouse.

The fifty sensitized mice were divided into five groups (10 mice/group) and treated as follows:

Group	N	Treatment
1	10	Sensitized, treated with vehicle (Dulbecco's phosphate buffered saline (PBS; Centocor, Inc., Malvern, PA)) - 10 ml/kg, intravenously (i.v.), 1 hour prior to and 24 and 48 hours post OA challenge.
2	10	Sensitized, treated with cV1q muG2a antibody - 1 mg/kg, i.v., 1 hour prior to and 24 and 48 hours post OA challenge.
3 ^a	10	Sensitized, treated with cV1q muG2a antibody, 10 mg/kg, i.v., 1 hour prior and 24 and 48 hours post OA challenge.
4	10	Sensitized, treated with dexamethasone (Sigma Chemical Co., St. Louis, MO) - 1 mg/kg, intraperitoneally (i.p.), 1 hour prior to and 24 and 48 hours post OA challenge.
5	10	Sensitized and challenged with 0.9% saline.

^a One animal died following first treatment with cV1q muG2a (10 mg/kg, i.v.)

Mice were challenged with OA by exposure to aerosolized OA on day 21 (5% w/v in sterile saline (Baxter, Inc., Chicago, IL)) for 20 minutes. The aerosol was generated by a PARI-Master nebulizer (PARI-Respiratory, Richmond, VA). The outlet of which was connected to a small Plexiglas® chamber (Pena-Plas, Jessup, PA) containing the animals.

On day 24, seventy-two hours following OA or saline aerosol exposure, animals were retroorbitally bled and serum was collected and frozen for total serum IgE analysis. Following bleeding, animals were anesthetized with urethane (0.2 g/kg) and bronchoalveolar lavage (BAL) was performed. Briefly, the trachea was exposed and cannulated. Lungs were lavaged with 2 x 0.5 ml sterile Hank's

balanced salt solution (HBSS; Gibco, Grand Island, NY) without Ca^{2+} and Mg^{2+} , containing 0.1% EDTA. Lavage fluid was recovered after 30 seconds by gentle aspiration and pooled for each animal. Samples were centrifuged at 2000 rpm for 15 minutes at 5°C. Individual pellets were reconstituted with 1 ml HBSS without Ca^{2+} and Mg^{2+} , containing 0.1% EDTA. BAL total cell and differential white cell (eosinophil) counts were determined using a Technicon H1 (Roche Diagnostics, Switzerland) and cytoslide, respectively.

The serum was separated from each sample and assayed for IgE antibodies by ELISA assay. Briefly, microtiter plates were coated with 100 μl of a monoclonal rat anti-mouse IgE antibody and incubated 1 hour (± 15 min) at 37°C ($\pm 2^\circ$) and overnight at 4°C ($\pm 2^\circ$). Plates were blocked with 300 μl 1% bovine serum albumin (BSA) for 1 hour (± 15 min) at 37°C ($\pm 2^\circ$). Plates were washed 5 times. Test serum was diluted 1:3, 1:6, 1:12, and 1:24 with 1% BSA in phosphate buffered saline plus 0.05% Tween-20 (PBST). 100 μl of the diluted sera was added to duplicate wells and incubated for 1.5 hours (± 15 min) at 37°C ($\pm 2^\circ$). The outside wells around the plate were not used to avoid perimeter effects. 100 μl rabbit anti-mouse IgE was added to each well and the plates incubated for 1.5 hours (± 15 min) at 37°C ($\pm 2^\circ$). 100 μl biotinylated goat anti-rabbit IgG was added to each well and the plates incubated for 1.5 hours (± 15 min) at 37°C ($\pm 2^\circ$). Streptavidin-conjugated horseradish peroxidase (100 μl) was added to each well and the plates incubated 15 minutes (± 2 min) at 37°C ($\pm 2^\circ$). Plates were washed five times with PBST between each incubation. TMB peroxidase substrate (100 μl) was added to each well and incubated at 37°C ($\pm 2^\circ$). 100 μl 1M phosphoric acid was added to each well to terminate the reaction. Absorbance was read at 450 nm using a UVMax Microplate reader from Molecular Devices Corporation (Sunnyvale, CA). A standard curve using a monoclonal mouse IgE anti-DNP (SPE-7) (Sigma Chemical Co., St. Louis, MO) was run with the assay.

Total cell, eosinophil and serum IgE levels from various treatment groups were compared using an ANOVA followed by a multiple comparison test (Zar, J.H., *Biostatistical Analysis*, Prentice Hall: Englewood, NJ, p. 185 (1984)).

Total Cell, Eosinophil and Serum IgE

BAL total cell, eosinophil and total serum IgE levels from the various treatment groups are shown in Table 1.

TABLE 1: Antigen-Induced Pulmonary Inflammatory Cell Accumulation in the Mouse Individual Animal Data

Group Number	Animal Number	Body Weight (g)	Total Cells ($\times 10^6/\text{ml}$)	EOS ^a ($\times 10^6/\text{ml}$)	EOS ^a (% of total)	Total Serum IgE (ng/ml)
5	1	22	0.87	0.50	57	328
	2	21	0.6	0.23	39	218
	3	21	2.19	1.20	55	243
	4	21	0.97	0.44	45	419
	5	21	0.47	0.14	30	305
	6	21	0.16	0.09	58	242
	7	20	0.80	0.48	60	292
	8	19	1.30	0.81	62	241
	9	19	0.28	0.12	44	366
	10	20	0.62	0.23	37	410
	11	21	0.68	0.22	33	159
	12	20	0.60	0.16	27	124
	13	22	0.55	0.05	9	134
	14	21	0.92	0.35	38	208
	15	15	0.79	0.04	5	312
	16	23	0.68	0.12	18	345
	17	22	0.55	0.14	25	116
	18	21	0.68	0.08	12	280
	19	20	0.68	0.13	19	250
	20	21	0.67	0.11	16	402

^aEOS = eosinophils

TABLE 1: Antigen-Induced Pulmonary Inflammatory Cell Accumulation in the
Mouse Individual Animal Data (continued)

Group Number	Animal Number	Body Weight (g)	Total Cells ($\times 10^6/\text{ml}$)	EOS ^c ($\times 10^6/\text{ml}$)	EOS ^c (% of total)	Total Serum IgE (ng/ml)
5	3	21	0.58	0.12	20	325
		22	0.67	0.01	2	269
		23 ^a	-	-	-	-
		24	0.06	0	4	361
		25	0.07	0.02	22	316
		26	0.69	0.01	1	374
		27	0.55	0.15	27	173
		28	0.47	0.06	13	130
		29	1.07	0.33	31	502
		30 ^b	0.02	-	-	502
	4	31	0.57	0.11	20	284
		32	0.24	0.01	5	553
		33	0.31	0.01	2	545
		34	0.80	0.32	40	106
		35	0.31	0.05	17	105
		36	0.53	0.09	17	254
		37	0.88	0.43	49	136
		38	0.73	0.16	22	191
		39	0.51	0.08	15	149
		40	0.45	0.01	2	154

^a Animal found dead one day following OA challenge

^b Animal not included in summary data

^cEOS = eosinophils

TABLE 1: Antigen-Induced Pulmonary Inflammatory Cell Accumulation in the Mouse Individual Animal Data (continued)

Group Number	Animal Number	Body Weight (g)	Total Cells ($\times 10^6/\text{ml}$)	EOS ^a ($\times 10^6/\text{ml}$)	EOS ^a (% of total)	Total Serum IgE (ng/ml)
5	41	19	0.76	0	0	184
	42	21	0.06	0	0	230
	43	19	0.33	0	0	157
	44	20	0.42	0	0	262
	45	20	0.61	0.01	1	275
	46	21	0.70	0.01	1	348
	47	18	0.50	0	0	176
	48	21	0.59	0	1	133
	49	20	0.54	0	0	119
	50	19	0.35	0.01	2	63

^aEOS = eosinophils

As illustrated in Figure 1, a 20 minute OA (5%) exposure to sensitized mice produced an approximate 2-fold increase in BAL total cells compared to saline challenged mice. Bronchoalveolar lavage eosinophils increased from virtually 0 in saline challenged mice to $0.42 \pm 0.11 \times 10^6$ 72 hours following OA challenge (Figure 1). The increase in BAL total cells 72 hours following OA challenge resulted primarily from the increase in eosinophils (Figure 2). As shown in Figure 3, total serum IgE levels increased by 56% following antigen challenge in sensitized mice compared to saline challenged sensitized mice.

The positive control, dexamethasone (1 mg/kg, i.p., a steroidal anti-inflammatory) administered 1 hour prior to and 24 to 48 hours following OA challenge inhibited antigen-induced increases in total cells and eosinophils by 36% and 69%, respectively, compared to the vehicle-treated group (Figure 1). Dexamethasone also produced a 30% reduction in total serum IgE levels compared to the vehicle-treated group (Figure 3).

Intravenous administration of cV1q muG2a antibody, an anti-TNF α monoclonal antibody, at 1 and 10 mg/kg 1 hour prior to and 24 and 48 hours following antigen challenge (OA challenge) produced a 18% and 37% reduction, respectively in total cells compared to the vehicle-treated group (Figure 1)

5 (0.52 \pm 0.09 x 10⁶/ml in the 10 mg/kg anti-TNF α treated group versus 0.83 \pm 0.18 x 10⁶/ml in the vehicle-treated group, NS). In addition, cV1q muG2a antibody administration at 1 and 10 mg/kg inhibited antigen-induced (OA-induced) increases in BAL eosinophils by 67% and 79%, respectively compared to vehicle-treated animals (Figure 1) (0.09 \pm 0.04 x 10⁶/ml in the 10 mg/kg anti-TNF α treated group

10 versus 0.42 \pm 0.11 x 10⁶/ml in the vehicle-treated group, p<0.05). These results indicate that anti-TNF α antibody modulates antigen-induced pulmonary inflammatory cell accumulation in sensitized mice.

In summary, intravenous administration of cV1q muG2a antibody at 1 and 10 mg/kg at 1 hour prior to and 24 to 48 hours following OA challenge produced a

15 67% and 79%, respectively, reduction in BAL eosinophils compared to vehicle-treated animals. Thus, treatment with anti-TNF α antibody resulted in a significant reduction in the number of total cells and eosinophils in BAL.

Pharmacokinetics

cV1q antibody concentrations in the serum samples were analyzed by

20 enzyme immunoassay (EIA). Briefly, a monoclonal anti-idiotypic antibody specific for the cV1q antibody (Lot SM970109; Centocor, Inc., Malvern, PA) was coated onto a 96 well microtiter plate. The plates were then washed and blocked with 1% bovine serum albumin (BSA)/phosphate buffered saline (PBS) solution to prevent non-specific binding. This blocking solution was removed. cV1q muG2a antibody

25 standards and diluted test samples were added to the plate for a 2 hour incubation. The plates were washed and a biotinylated version of a different anti-cV1q monoclonal antibody was added to all wells for a 2 hour incubation. The plates were washed and incubated with a horseradish peroxidase-streptavidin conjugate during a third incubation period. A final enzymatic color development step was performed

30 using o-phenylenediamine (Sigma Chemical Co., St. Louis, MO) as a substrate.

Color development was stopped with the addition of 4N sulfuric acid and the light absorbency read using a microtiter plate spectrophotometer at 490 nm. The cV1q antibody standard concentrations and their corresponding optical density values were used to construct a standard curve by a computer generated least squares fit to a four parameter equation. Sample cV1q antibody concentrations were then determined using the standard curve and the serum dilution factor for that sample.

Results

cV1q antibody concentrations in the serum and BAL samples from the mice treated with 1 and 10 mg/kg of cV1q antibody are shown in the upper and lower sections, respectively, of Table 2.

TABLE 2: Serum and BAL cV1q Antibody Concentrations ($\mu\text{g/ml}$)

cV1q muG2a Antibody (1 mg/kg, i.v.)											
Mouse	11	12	13	14	15	16	17	18	19	20	Mean \pm SD
Sera	29.7	28.5	37.6	23.8	23.4	26.7	21.0	31.2	21.4	27.8	27.1 \pm 5.06
BAL	.042	.055	<0.04	.069	.118	.062	.055	.071	.119	.076	.067 \pm .035
cV1q muG2a Antibody (10 mg/kg, i.v.)											
Mouse	21	22	23	24	25	26	27	28	29	30	Mean \pm SD
Sera	317	282	NS	295	402	289	301	291	257	284	302 \pm 40.8
BAL	1.65	.537	NS	.626	.176	.391	.429	.306	.851	<0.04	.55 \pm .48

NS = No Sample

Serum and bronchiolar lavage (BAL) samples from the vehicle control group (n=10) had no detectable levels of cV1q muG2a (cV1q) antibody (<0.04 $\mu\text{g/ml}$). Following multiple (n=3) intravenous administrations of cV1q antibody at 1 mg/kg, the serum samples from these antibody treated mice (n=10) had a mean \pm standard deviation cV1q antibody concentration of 27.1 \pm 5.06 $\mu\text{g/ml}$; the BAL samples from these mice had a mean cV1q antibody concentration of 0.067 \pm 0.035 $\mu\text{g/ml}$. The mean serum cV1q antibody concentration (n=9) following multiple (n=3)

intravenous administrations of 10 mg/kg of the antibody, was $302 \pm 40.8 \mu\text{g/ml}$; the mean cV1q antibody concentration of the BAL samples from these mice was $0.55 \pm 0.48 \mu\text{g/ml}$.

5 The determined concentrations of cV1q antibody from the serum and BAL mouse samples confirm a dose dependent treatment with anti-TNF α antibody and that the antibody can be detected in BAL following an intravenous administration.

EXAMPLE 2 Antigen-Induced Pulmonary Inflammatory Cell Accumulation In The Mouse: Histopathological Evaluation.

10 A histopathological evaluation was performed on the lungs from sensitized female Balb/CJ mice.

Experimental Procedure

Twenty female Balb/CJ mice were sensitized at weeks of age by intraperitoneal injections of 10 μg OA (Sigma Chemical Co., St. Louis, MO) mixed in 1.6 mg aluminum hydroxide gel suspension (Intergen, Inc., Purchase, NY) in
15 0.2 ml sterile saline on days 0, 7 and 14. This suspension was prepared one hour before intraperitoneal injection into each mouse.

The twenty sensitized were divided into two groups (10 mice/group). One group of mice was administered intravenously 10 mg/kg cV1q muG2a antibody (Group 2) 1 hour prior to and 24 and 48 hours following OA challenge. The other
20 group of mice was administered intravenously 10 ml/kg Dulbecco's PBS (Centocor, Inc., Malvern, PA) (vehicle) (Group 1) 1 hour prior to and 24 and 48 hours following OA challenge. Mice were challenged with OA (antigen) by exposure to aerosolized on day 21 (5% w/v in sterile saline (Baxter, Inc., Chicago, IL) for 20 minutes. The aerosol was generated by a PARI-Master nebulizer (PARI-Respiratory, Richmond,
25 VA). The outlet of which was connected to a small Plexiglas® chamber (Pena-Plas, Jessup, PA) containing the animals.

Seventy-two hours following antigen challenge, the mice were sacrificed and the lungs were removed and filled with 10% neutral buffer formalin (NBF; Sigma Chemical Co., St. Louis, MO). Lungs were then embedded in paraffin and stained

with hematoxylin and eosin. The microscopic changes were graded on a scale of one to four (minimal, slight/mild, moderate and marked/severe) depending upon the severity of the change.

Results

- 5 Microscopic changes which could not be graded were designated as Present (P). All of the microscopic findings are presented in Table 3.

TABLE 3: Microscopic Changes In the Lungs Of the Mice

Group/Treatment		Group 1									
Animal Number		1	2	3	4	5	6	7	8	9	10
10	LUNGS*										
	Perivascular Leukocytes	2	2	3	3	3	2	3	2	2	2
	Perivascular Edema	1			1	1	2	2			1
	Mineralized Vessel, Focal										
	Interstitial Leukocytes	1	2	2	2	2	1	2	2	2	1
15	Interstitial Eosinophilic Deposits	1	1	1	1	2	1	1	2	1	2
	Alveolar Leukocytes	1	1		2	2	1	1	1	1	1
	Alveolar Macrophages	1	1			1				1	
	Alveolar Hemorrhage					2					
20	Pleural Leukocytes	2	2	2	2	3	1	2	2	2	1
	Pleural Macrophages										
	Peribronchial Lymph Node										
	Eosinophilic Macrophages										

* SEVERITY CODE: 1 = MINIMAL, 2 = SLIGHT, 3 = MODERATE, 4 = SEVERE,

- 25 P = PRESENT

TABLE 3: Microscopic Changes In the Lungs Of the Mice (continued)

Group/Treatment		Group 2									
Animal Number		1	2	3	4	5	6	7	8	9	10
LUNGS*											
5 Perivascular Leukocytes		2	2	1	1	1	1	1	1	2	2
Perivascular Edema		1			1					1	
Mineralized Vessel, Focal				P							
Interstitial Leukocytes		1	1			1					
10 Interstitial Eosinophilic Deposits		2	1			1	1	2	1		
Alveolar Leukocytes		1	1			1				1	1
Alveolar Macrophages					2	1		1		1	1
Alveolar Hemorrhage											2
Pleural Leukocytes		2	2			1				2	2
15 Pleural Macrophages											4
Peribronchial Lymph Node											
Eosinophilic Macrophages							3				4

* SEVERITY CODE: 1 = MINIMAL, 2 = SLIGHT, 3 = MODERATE, 4 = SEVERE,

P = PRESENT

20 Inflammatory cell accumulations were present and enumerated in three areas of the lungs of individual mice in both test groups. Leukocyte accumulations were evaluated in the perivascular tissues surrounding the vessels in the bronchial areas, the interstitial tissues of the alveolar areas and in the pleural/subpleural tissues. A few mice in both groups had perivascular edema around the vessels in the bronchial

25 areas. Individual mice in both groups had eosinophilic fibrin-like deposits in the capillaries of the interstitial tissues. Group 2 mice numbered 6 and 10 had moderate and severe, respectively, accumulations of eosinophilic staining macrophages in the peribronchial lymph nodes. Group 2 mouse number 10 also had severe accumulations of eosinophilic staining macrophages in the pleural tissues and

30 peribronchial tissues admixed with inflammatory cells.

As a group, when compared to Group 1 (vehicle-treated mice), histopathological analysis showed significant reduction in the number of perivascular leukocytes, interstitial leukocytes and pleural leukocytes in the mice in Group 2 (cV1q-treated mice). These results show that anti-TNF α antibody
5 modulates antigen-induced pulmonary inflammatory cell accumulation in sensitized mice.

EXAMPLE 3 Infiximab Therapy For Steroid Resistant Asthma.

A 53 year old woman (N.L.) with mild chronic obstructive pulmonary disease and severe steroid dependent asthma, developed worsening of asthma over
10 several weeks despite intensive treatment with 40 mg of prednisone orally, inhaled steroids, inhaled ipratropium, inhaled albuterol, inhaled salmeterol, oral theophylline and zileuton. Side effects from this substantial but ineffective program included weight gain, skin thinning, and bruising.

Treatment with infliximab was instituted according to Table 4.

15 TABLE 4: Infiximab Infusion (Patient N.L.)

Day	Infusion Number	Infusion Dose (mg)	Cumulative Dose (mg)
0	1	200	200
4	2	200	400
16	3	400	800
20 45	4	400	1,200

The patient received four infusions totaling 1,200 mg of infliximab during the treatment period.

Results

There was a decline in asthma symptoms, cessation of nighttime awakening,
25 a reduction in steroid use, and less reliance on inhaled medication. This

improvement began within 24 hours of infliximab therapy and is documented in Table 5, the patient's diary card.

TABLE 5: Diary Card

	Day	Asthma Symptoms Over Past 24 Hours*	Number of Times You Woke Up Last Night Due to Asthma	Number of Puffs of Proventil Used In the Last 24 Hours	Number of Nebulization Treatments Used In the Last 24 Hours	Steroid Use (Total Dose Daily) (mg)	Peak Flow Score (ml/min)**	
							AM	PM
5	2	4	1	6	4	20	200	160
	3	2	0	0	2	15	200	400
	4	2	0	0	2	15	205	400
	5	2	0	0	2	10	275	400
	6	2	0	2	2	10	255	400
10	7	2	0	0	2	0	200	400
	8	2	0	0	1	10	205	400
	9	2	0	0	2	0	205	400
	10	2	0	0	2	10	200	400
	11	2	0	2	2	0	195	400
15	12	2	0	0	2	10	195	400

* Asthma Symptom Scores were done each morning using the following scale:

- 0 = No symptoms during the day
 1 = Symptoms for one short period during the day
 2 = Symptoms for two or more short periods during the day
 3 = Symptoms for most of the day which did not affect your normal daily activities
 4 = Symptoms for most of the day which did affect your normal daily activities
 5 = Symptoms so severe that you missed work or could not perform normal daily activities

** Peak flow scores were measured using a pediatric flow meter or both a pediatric flow meter (P) and an adult flow meter (A), as indicated.

TABLE 5: Diary Card (continued)

Day	Asthma Symptoms Over Past 24 Hours*	Number of Time You Woke Up Last Night Due to Asthma	Number of Puffs of Proventil Used In the Last 24 Hours	Number of Nebulization Treatments Used In the Last 24 Hours	Steroid Use (Total Dose Daily) (mg)	Peak Flow Score (ml/min)**	
						AM	PM
13	2	0	0	2	0	245	400
14	2	0	0	1.5	10	225	400
15	2	0	0	2	0	245	400
16	2	0	0	2	10	200	400P/350A
17	2	0	0	2	0	180P/160A	400/310
18	2	0	0	2	10	220/200	400/320
19	2	0	0	2	0	370/225	400/320
20	2	0	0	2	10	370/270	400/340
21	2	0	0	2	0	305/260	400/330
22	2	0	0	2	10	230/200	400/350
23	2	0	0	2	0	205/240	400/330
24	2	0	0	2	10	250/210	400/340
25	2	0	0	2	0	220/200	400/350
26	2	0	0	2	10	175/200	400/355
27	2	0	0	2	0	200/210	400/350
28	2	0	0	3	10	235/210	400/350

* Asthma Symptom Scores were done each morning using the following scale:

- 0 = No symptoms during the day
 1 = Symptoms for one short period during the day
 2 = Symptoms for two or more short periods during the day
 3 = Symptoms for most of the day which did not affect your normal daily activities
 4 = Symptoms for most of the day which did affect your normal daily activities
 5 = Symptoms so severe that you missed work or could not perform normal daily activities

** Peak flow scores were measured using a pediatric flow meter or both a pediatric flow meter (P) and an adult flow meter (A), as indicated.

TABLE 5: Dairy Card (continued)

Day	Asthma Symptoms Over Past 24 Hours*	Number of Time You Woke Up Last Night Due to Asthma	Number of Puffs of Proventil Used In the Last 24 Hours	Number of Nebulization Treatments Used In the Last 24 Hours	Steroid Use (Total Dose Daily) (mg)	Peak Flow Score (ml/min)**	
						AM	PM
5	29	2	0	0	2	0	225/200
	30	4	0	0	3	10	200/170
	31	2	0	0	2	0	180/180
	32	2	0	0	2	10	225/190
	33	1	0	0	2	0	275/250
10	34	1	0	0	2	10	210/240
	35	2	0	0	2	0	300/200
	36	2	0	0	2	10	230/220
	37	1	0	0	2	0	275/250
	38	1	0	0	2	10	210/190
15	39	1	0	0	2	0	245/180
	40	1	0	0	2	10	195/180
	41	1	0	0	2	0	180/170
	42	3	0	0	2	10	230/210
	43	3	0	0	2	0	235/210
	44	2	0	0	2	10	190/170
	45	-	0	-	-	0	175/180

20 * Asthma Symptom Scores were done each morning using the following scale:

- 0 = No symptoms during the day
 1 = Symptoms for one short period during the day
 2 = Symptoms for two or more short periods during the day
 3 = Symptoms for most of the day which did not affect your normal daily activities
 25 4 = Symptoms for most of the day which did affect your normal daily activities
 5 = Symptoms so severe that you missed work or could not perform normal daily activities

** Peak flow scores were measured using a pediatric flow meter or both a pediatric flow meter (P) and an adult flow meter (A), as indicated.

Peak flow score is the highest velocity of air flow recorded for the patient as measured in a breathing test. In contrast to pre-treatment peak flow scores of 160 to 200 ml/min, peaks of 340 to 400 ml/min were recorded during the infliximab treatment schedule. Higher peak flow scores are better than lower scores.

5 During infliximab treatment, inhaled albuterol was not required. In addition, steroid use was reduced to 10 mg every other day.

The patient's quality of life was improved greatly when she received infliximab. For example, comparing the patient's quality of life responses, the patient's asthma became well controlled, and awakening at night had disappeared
10 after the second day of infliximab treatment.

Table 6 shows the objective improvement in pulmonary function studies.

TABLE 6: Pulmonary Function Tests (Patient N.L.)

Day	Forced Voluntary Capacity (FVC)	Forced Expiratory Volume in 1 second (FEV ₁)	FEV ₁ /FVC	Forced Expiratory Flow (FEF 25-75)
15 2 (Baseline)	54%	33%	56%	13%
16 (Treatment)	82%	60%	73%	25%
22 (Treatment)	71%	50%	57%	22%
20 28 (Treatment)	79%	55%	57%	23%
36 (Treatment)	87%	66%	61%	32%
25 45 (Treatment)	80%	54%	67%	22%

Forced voluntary capacity (FVC) is a measure of expiratory flow. Forced expiratory volume in 1 second (FEV₁) is the maximum amount of air that can be blown out by the patient in 1 second. Forced expiratory flow (FEF 25-75) is a velocity measurement between the first and third quarter of 1 second. Higher values

are better than lower values. The FEV₁ values observed were the highest documented for the patient during her care in about two years.

This 53 year old female patient had prompt and sustained improvement in both signs and symptoms of treatment resistant asthma during infliximab therapy.

- 5 Infliximab therapy reduced or eliminated the need for poorly tolerated or ineffective therapies.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without
10 departing from the spirit and scope of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. Use of an anti-TNF α antibody or an antigen-binding fragment thereof for the manufacture of a medicament for use in the treatment of asthma or airway
5 inflammation in an individual in need thereof.
2. Use of an anti-TNF α antibody or an antigen-binding fragment thereof for the manufacture of a medicament for use in reducing accumulation in lungs of inflammatory cells in an individual in need thereof.
3. Use according to Claims 1 or 2, wherein the antibody is a chimeric antibody.
- 10 4. Use according to Claim 3 wherein the chimeric antibody competitively inhibits binding of TNF α to the cA2 monoclonal antibody.
5. Use according to Claim 3 wherein the chimeric antibody is the cA2 monoclonal antibody.
6. A method of treating asthma in an individual comprising administering to the
15 individual a therapeutically effective amount of an anti-TNF α antibody or an antigen binding fragment thereof.
7. The method of Claim 6 wherein the antibody is a chimeric antibody.
8. The method of Claim 7 wherein the chimeric antibody competitively inhibits binding of TNF α to the cA2 monoclonal antibody.

9. The method of Claim 7 wherein the chimeric antibody is the cA2 monoclonal antibody.

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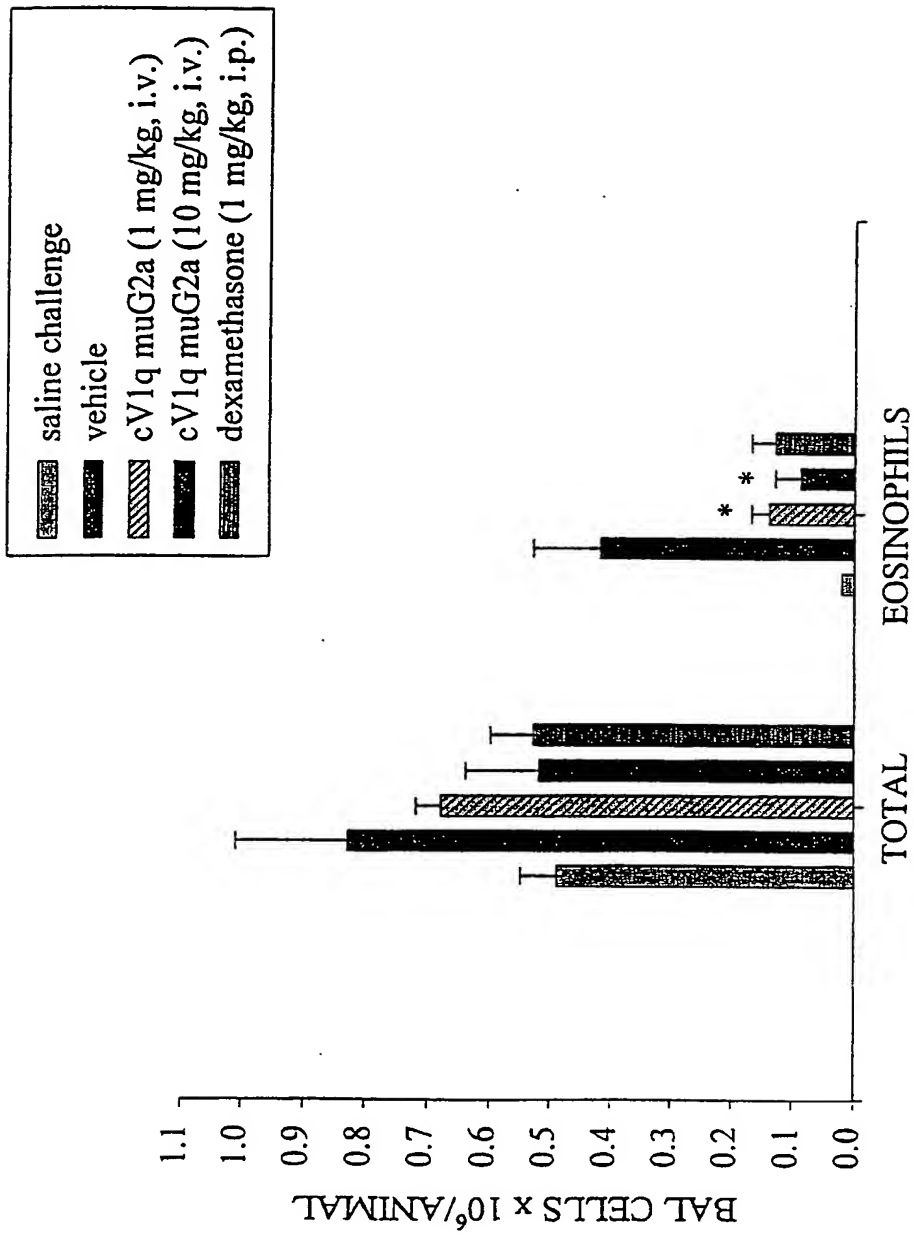


Fig. 1

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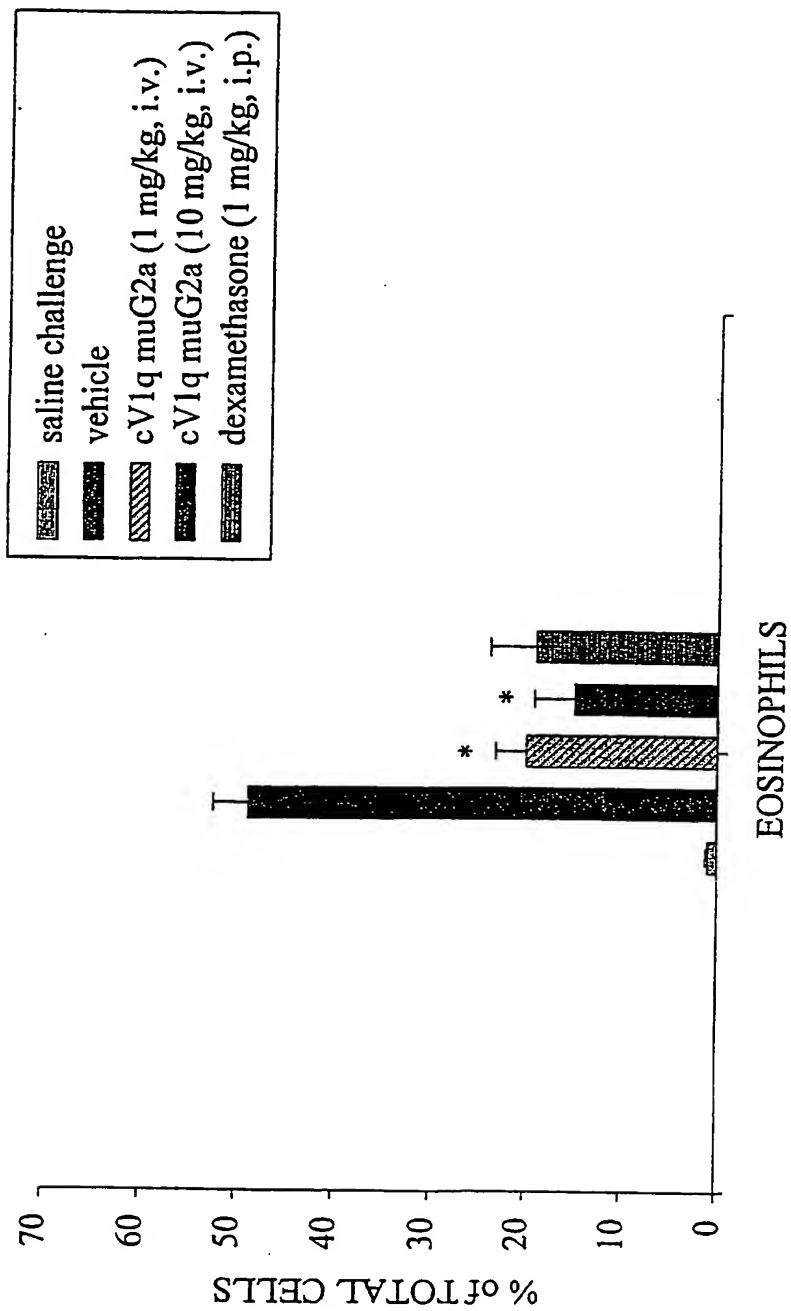


Fig. 2

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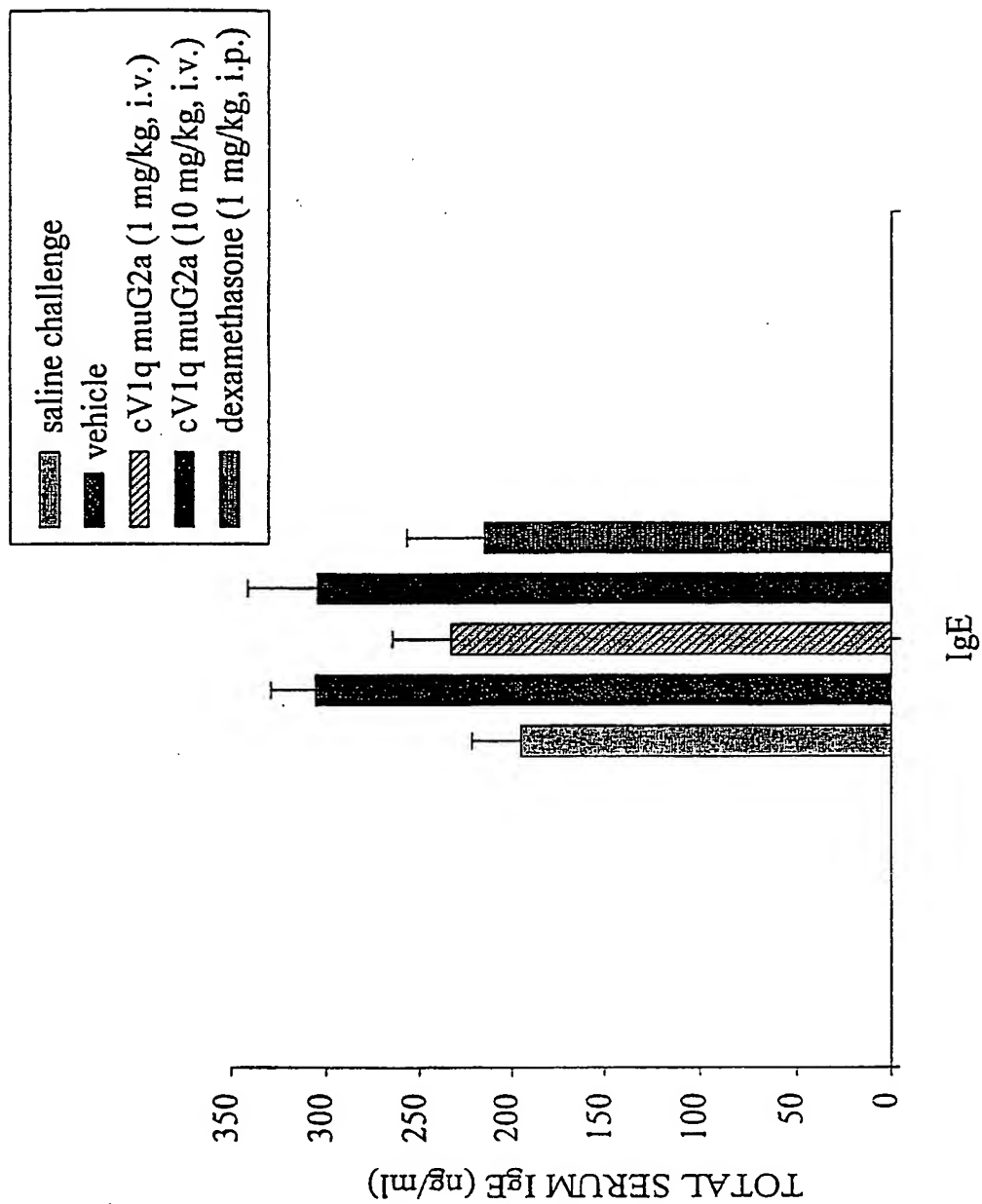


Fig. 3

INTERNATIONAL SEARCH REPORT

In .ational Application No

PCT/US 00/05163

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 04054 A (A. MILLAR) 4 April 1991 (1991-04-04) the whole document ---	1-9
X	DE 41 39 733 A (SCHERING AG) 3 June 1993 (1993-06-03) column 1, line 1 - line 39 ---	1-9
X	WO 92 08474 A (THE NATIONAL HEART & LUNG INSTITUTE) 29 May 1992 (1992-05-29) page 27, line 4 -page 28, line 11 claims 1,8,11,18,22,23 ---	1-9
X	WO 98 46642 A (FARMACEUTISK LABORATORIUM FERRING A/S) 22 October 1998 (1998-10-22) claims 27,28,33 ---	1-9
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

Date of the actual completion of the international search

7 June 2000

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

In. .ational Application No

PCT/US 00/05163

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International Application No

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